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Discovery of Selective Nanobodies against α -elapitoxin Dpp2c from Black Mamba through Phage Display Screening

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Targeting black mamba α -neurotoxins with nanobodies

Fearful for its highly neurotoxic venom and rapid attack technique, the Black mamba (*Dendroaspis polylepis*) is Africa's largest venomous snake [1]. The clinical manifestations of a bite from *D. polylepis* include flaccid paralysis leading to respiratory failure and death due to post-synaptic blockade of the neuromuscular junctions caused by α -neurotoxins [1-4]. Since antivenoms suffer from a reactivity bias towards larger toxins due to the fact that antivenoms are produced by immunization of large mammals, current antivenoms could be reinforced by addition of monoclonal antivenoms directed towards the smaller α -neurotoxins [1,5]. Here, we report the discovery of selective nanobodies targeting α -elapitoxin Dpp2c from *D. polylepis* through phage display screening [6].



Figure 1: *Dendroaspis polylepis* (Black mamba) eating prey. Photo: Ted Arensmeier 2007

Results – two selective nanobody binders discovered

A M13 phage library displaying nanobody genes from a llama immunized with venom from the monocled cobra (*Naja kaouthia*) [7,8] was selected against a venom fraction from *D. polylepis* venom containing high amounts of α -elapitoxin Dpp2c. Two monoclonal phages that bound strongly to this fraction were isolated. Monoclonal phage DNA will soon be sequenced, which will unveil the primary structure of the nanobodies displayed on the phages.

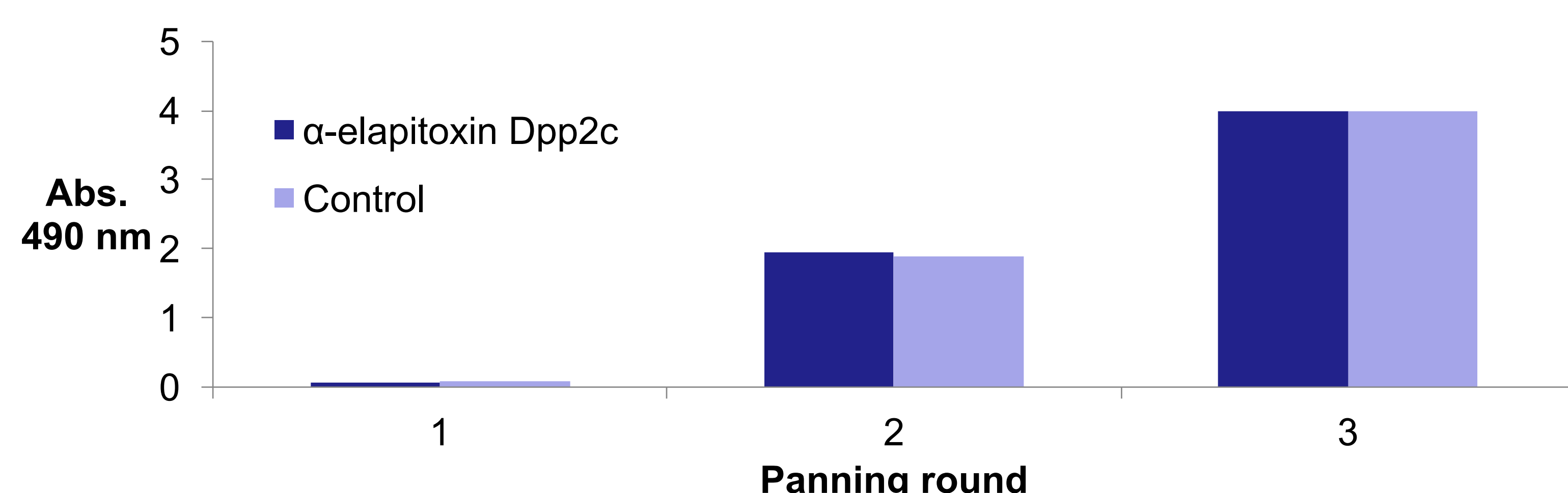


Figure 2: ELISA results. The polyclonal phage library from the third round of panning yields a strong ELISA signal, indicating the presence of strong peptide binders to α -elapitoxin Dpp2c

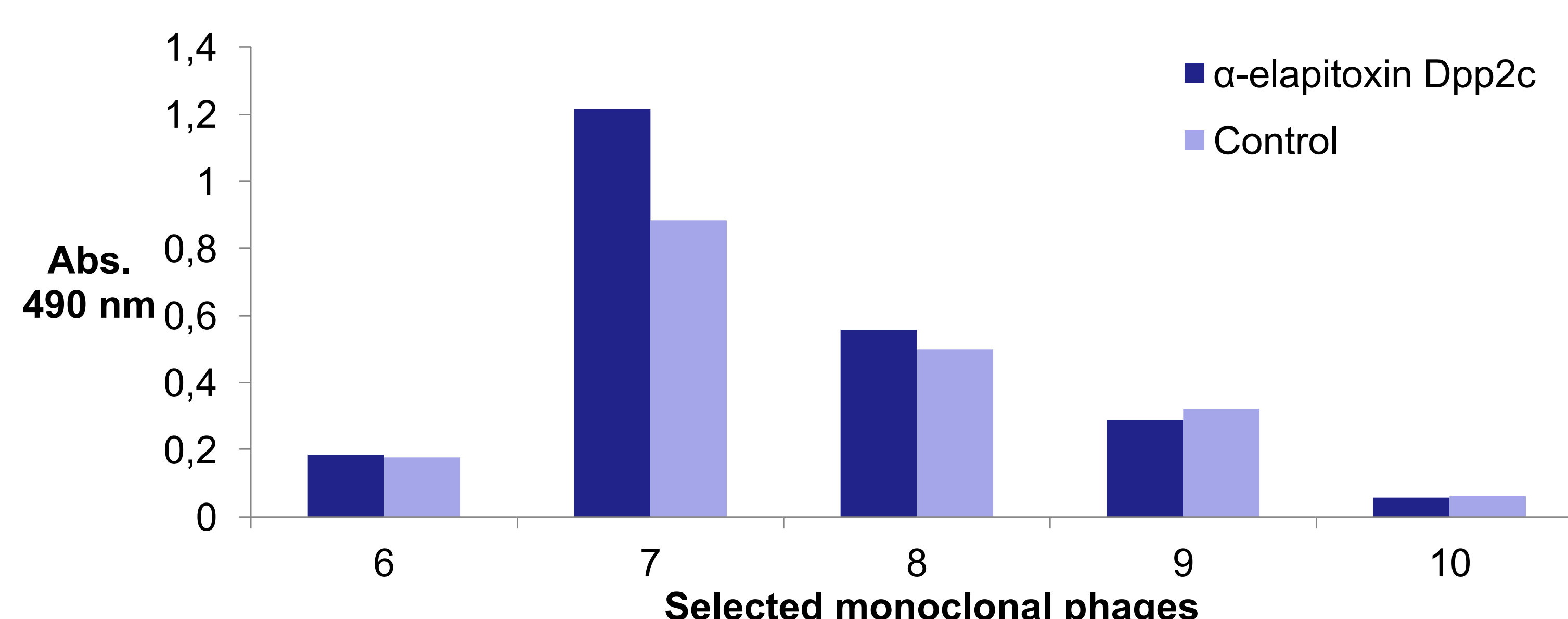


Figure 3: ELISA results for 10 selected phage monoclonal phages against α -elapitoxin Dpp2c.

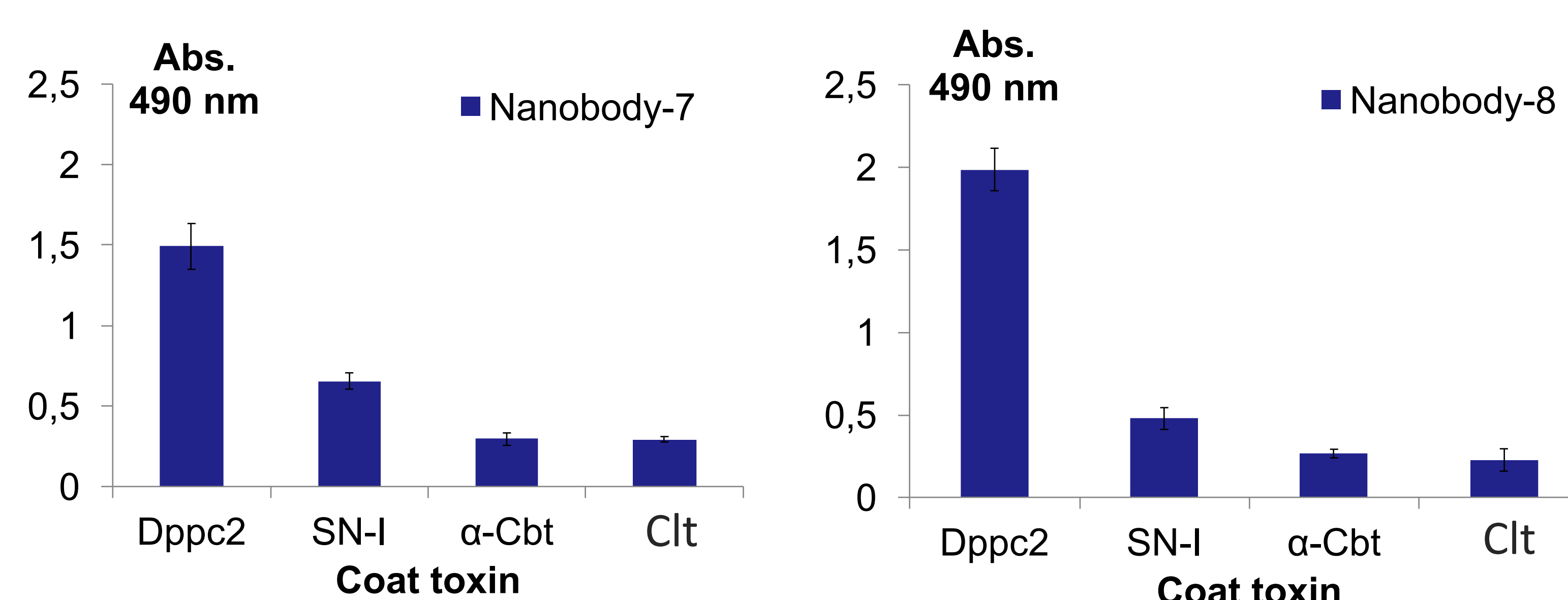


Figure 4: ELISA-based cross-reactivity study of the nanobody-displaying isolated phages. Dppc2: α -elapitoxin Dpp2c, SN-I: Short neurotoxin I, α -Cbt: α -cobratoxin.

Outlook – Reinforcing antivenoms with nanobodies

The isolated monoclonal nanobody displaying phages showed great selectivity towards α -elapitoxin Dpp2c and could potentially be added to existing antivenoms to reinforce their response towards this lethal α -neurotoxin. Once the sequences of the displayed nanobodies is known, the next steps include biosynthesis, determination of binding constants for the nanobodies, and measurement of their ability to inhibit α -elapitoxin Dpp2c *in vitro* and *in vivo*.

α -elapitoxin Dpp2c Overlay of α -elapitoxin Dpp2c and α -Cbt



SNT-I | RICYNHQSTTRATTKSC--ENSCKYKKYWRD----HRGTIIERGCG--CPKVKPG-VGIHCCQSDKCN----- 60
Dpp2c | RTCKNTPSDQ---SKICPPGENICYTKTWCDAWCSQRGKIVELCAATCPKVKAG-VEIKCCSTDNCKEKFEGKPR 72
MT- α | LTCVTSKSIIFGITTENPDGQNLCKFKWYYLN---HRYSDITWGCATCPKPTNVRETIHCCETDKCNE----- 66
* . . * : : * : * : * : : * . : : * : * : * : * : * : * : * : *

Figure 5: Cartoon model of α -elapitoxin Dpp2c compared with overlaid cartoon models of α -elapitoxin Dpp2c and α -cobratoxin from *Naja kaouthia* (α -Cbt, cyan), drawn in PyMOL. Sequence alignment short neurotoxin I (SNT-I), α -elapitoxin Dpp2c (Dpp2c), and muscarinic toxin- α (MT- α), which are all α -neurotoxins from *D. polylepis*.

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